(2)

EAST CAROLINA UNIVERSITY

School of Medicine

Department of Clinical Pathology
and Diagnostic Medicine
1S-08 Brody Medical Sciences Building

919-551-2356

Department of Pathology
Pitt County Memorial Hospital

919-551-4495



QUARTERLY REPORT (First Quarter, 3rd Year)
May 27, 1991

ONR Grant No. N00014-89-1712

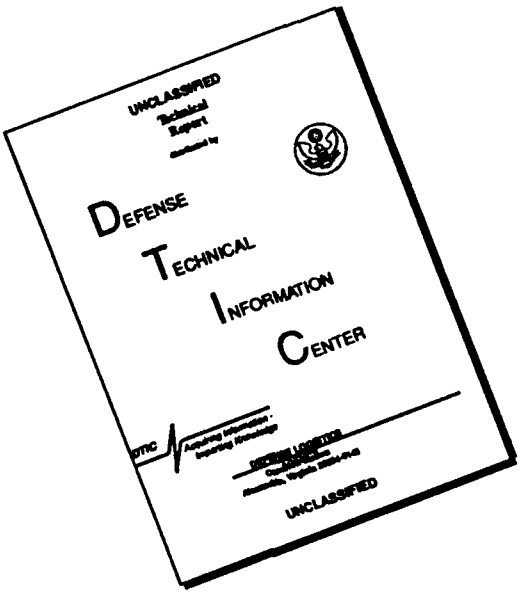
# Evaluation of Dried Storage of Platelets and RBC for Transfusion: Lyophilization and other Dehydration Techniques

Principal Investigator: Arthur P. Bode, PhD
East Carolina University
School of Medicine



Greenville,
North Carolina
27858-4354

# DISCLAIMER NOTICE




THIS DOCUMENT IS BEST QUALITY AVAILABLE. THE COPY FURNISHED TO DTIC CONTAINED A SIGNIFICANT NUMBER OF PAGES WHICH DO NOT REPRODUCE LEGIBLY.

## Project Summary

At the present time, the focus of research activity is on the in vivo animal model studies testing the participation of labelled lyophilized platelets in generation of experimentally-induced thrombi and testing of their circulatory lifespan. These studies require a considerable amount of preparation and analysis in order to establish significance of data among the natural variability of live systems. However, the preliminary reports from the investigators at UNC-Chapel Hill have been very encouraging. The development of alternative stabilization protocols, such as the permanganate experimentation at ECU and the rehydration technique development at the Tidewater Region worksite have been subordinated to the in vivo experimentation.

## Patents and Publication

An informal invention disclosure statement has been filed with the UNC-Chapel Hill patent committee in cooperation with similar office at ECU. After these administrative councils have evaluated the suitability of a patent application on the research products produced to-date, an official notice of support or release of rights will be forwarded to the ONR for consideration of appropriateness. Such notification is expected before July 1, 1991.

The manuscript on experimental results of the first two years of the grant is being withheld from submission until the question of pursuing a patent application has been answered by all involved parties. An abstract without data constituting full disclosure was presented by Dr. Read and colleagues at the FASEB meetings in Atlanta, GA, last month (April). See attached report summary from UNC-CH for further details. The presentation of data in the forum of a scientific conference allows for an assessment of interest in the project. Further abstracts will be submitted on future progress for meetings this Fall, including the annual meeting of the American Society of Hematology. A second manuscript will then also be prepared.

In addition, a grant proposal for continuation of these studies is in preparation. The ultimate goal is to enter preclinical trials of toxicity and efficacy of human blood cells stored in the dried state. The data produced from current project activity is supportive of the application of our initial findings to practical blood banking, based on a clear scientific understanding of the processes employed (however, empirically developed) to prevent cell damage during dehydration/rehydration. Much more needs to be investigated to ascertain the extent of involvement of dried platelets in thrombus formation and initiation and to establish the practicality of the preparations developed so far. A major new emphasis needs to be initiated on application of the successful techniques to preparation of dried human red cells for studies in circulation. This new grant proposal will be constructed on the knowledge base being produced during the currently-funded grant period, as well as extend into a more fully-developed examination of the specific effects of dehydration/rehydration on preservation of cell function.

Sincerely yours,

Arthur P. Bode, PhD
Principal Investigator

encl: report summary from UNC-CH

Quartly Report

Dehydration of Platelets and RBC: Long Term Storage of Transfusion Products.

UNC/ECU Contract, Grant No. N00014-89-J-1712 From The Office of Navel Research Department of The Navy.

Performance Site, University of North Carolina, Chapel Hill, N.C.

Marjorie S. Read, Ph.D., P.I.

Robert Reddick, M.D., Co. P.I.

Submitted to Dr. Arthur Bode, East Carolina University, May 7, 1991.

A. Ongoing studies with lyophilized platelets.

1. We are continuing to refine the dried platelet procedure and to study the effect of drying and rehydration on the in vivo performnce of the rehydrated cell.

2. Enclosed is the abstract from the poster which I presented at the FASEB meetings in Atlanta, Ga. last month. As you can see the work has gone very well. The rehydrated platelets are being tested in animals and have been observed in the circulation at 24 hours.

B. New studies conducted during this quarter.

1. We are looking at the hemostatic and thrombotic properties of the circulating, rehydrated platelets. In vivo animal studies are required to obtain these data. The pig and the dog are being used as animal models since each animal has unique characteristics that need to be studied.

2. We are looking at the release of vWF and other platelet proteins contained in the alpha granules. These studies reflect the ability of the stabilized platelet to be disrupted and to undergo fusion and loss of membrane intergrity.

C. Brief plans for the year.

We will continue to examine and refine protocols for in vivo use of rehydrated platelets.

# STUDIES WITH DRIED AND REHYDRATED PLATELETS FOR TRANSFUSION PRODUCTS

M.S. READ*, A.P. BODE† and R.L. REDDICK*

* Depts. of Pathology, University of North Carolina, Chapel Hill, NC
and † East Carolina University, Greenville, NC

SOCIETY OFFICE BY MONDAY, DECEMBER 3, 1990.

Mail to your Society of membership
(APS, ASBMB, ASPET, AAP, AIN, AAI)
ASCB members send to ASBMB
BMES and SEBM members send to APS
CIS, SMI, AAVI members send to AAI
ISB members to AAP

9650 Rockville Pike
Bethesda, MD 20814

# 1991 FASEB

# ABSTRACT FORM

**DO NOT FOLD THIS FORM**

MAILING ADDRESS OF FIRST AUTHOR
(Please print in black ink or type. Provide full name rather than initials.)

Marjorie S. Read
820 Brinkhous-Bullitt Bldg
UNC-CH Department Pathology
Chapel Hill, NC 27599

Phone: Office (919) 966-3146
Home/Holiday 489-3500

PRESENTATION PREFERENCE (Check one)

☐ Oral ☒ Poster ☐ Indifferent

Final decision regarding presentation format is at the discretion of the programing society.

SELECT CATEGORY NUMBERS & TITLES
(See Topic Category Lists)

1 629-4 Platelets
2 ____
3 ____

Is first author graduate student? ☐ Yes ☒ No

**IMPORTANT**

1. Prepare abstract carefully, on this form only.
2. Also fill out:
   a. Topic category selection
   b. Mailing address of first author
   c. Signature block for member's signature
3. Return to your society of membership by December 3, 1990
   a. The original typed abstract form
   b. Two photocopies
   c. Abstract handling fee ($30)
   d. Program confirmation card
4. See over for complete instructions.

REMITTANCE (NONREFUNDABLE)
ABSTRACT HANDLING FEE $30
Payable to FASEB

**Studies with Dried and Rehydrated Platelets for Transfusion Product.**

M.S. Read*, A.P. Bodet and R.L. Reddick*, Depts of Pathology, *University of North Carolina, Chapel Hill, NC 27599, †East Carolina University, Greenville, NC 27858.

Dried-rehydrated platelets are being studied for use as a substitute for fresh platelets in transfusion. Washed platelets from three species (human, canine and porcine) were treated with aldehydes prior to freezing and drying. The rehydrated platelets were compared to fresh platelets for adhesiveness and spread characteristics, *ex vivo*. The ability of the rehydrated platelets to circulate *in vivo* was also studied. Canine platelets were stabilized with paraformaldehyde (para) and glutaraldehyde (glut) and dried. Dried-rehydrated platelets were layered on formvar-coated grids and observed by scanning electron microscopy. Rehydrated platelets were compared to fresh platelets for morphologic evidence of pseudopod formation and platelet spread characteristics. Both fresh and rehydrated para-treated canine platelets had multiple pseudopodia and normal spread configurations. Glut-treated platelets had fewer pseudopods and few spread platelets were seen. In an *ex vivo* annular perfusion chamber, porcine and human, but not canine, fresh and para-treated platelets adhere at high sheer rates in near equal numbers to exposed subendothelium. The ability of rehydrated canine platelets to remain in circulation *in vivo* was studied using rehydrated para-treated canine platelets labeled with a fluorescent dye (Synaxis, PKH26-GL) infused into a dog. Blood samples were collected at various time intervals for 2 hours following platelet infusion. Smears made from whole blood and platelet-rich plasma aliquots were examined for fluorescence. Labeled para-treated platelets were found in the blood samples for up to 2 hrs post infusion. These studies suggest that fixed, rehydrated platelets can be prepared which will adhere to vascular subendothelium, and remain in the circulation for a period of time sufficient to aid in hemostasis. (Aided by ONR N00014-89-J-1712)

Blue lines are printer's cut lines, do not type on or outside of these lines

*All compounds that are designated by code or initial letters in the title must be identified adequately in the abstract,* e.g., MJ-1999; 4-(2-isopropylamino-1-hydroxyethyl) methanesulfonanilide hydrochloride.

**All investigators must adhere to the guidelines as listed on the reverse of the abstract form.**

**APS, BMES, SEBM, ASPET & AIN members only:**
Signing member, are you willing to chair a session? ☐ Yes, category # ____

**APS, AAP & AIN members only:**
☐ Submitted for special Society student or training award Type of award ____

**MEMBER'S AFFILIATION** (Check one only):
☐ APS ☐ ASBMB ☐ ASPET ☒ AAP ☐ AIN ☐ AAI ☐ ASCB ☐ BMES ☐ SEBM ☐ CIS ☐ SMI ☐ AAVI ☐ ISB

**Submission of signed form indicates acceptance of rules including final withdrawal date of December 28 1990. No exceptions will be made.**

Marjorie S. Read, PhD
Member's Name (Print or type)

Marjorie S. Read
Member's Signature

(919) 966-3146
Member's Phone